Protein diversity of *Paulownia* plant leaves and clusters

FAN Guo-qiang, PENG Hai-feng, ZHAI Xiao-qiao
(Institute of Panlownia, Henan Agricultural University, Zhengzhou 450002, P. R. China)

Abstract: *Paulownia tomentosa, P. fargesii, P. lamprorhylla, P. albiphloea, P. australis, P. fortunei, P. elongata, P. elongata f.alba* and *P. albiphloea* var *chenggtuensis* were classified into three groups: *P. fortunei* group (*P. fortunei* and *P. elongata f. alba*); *P. australis* group (*P. australis* and *P. albiphloea* var *chenggtuensis*) and *P. tomentasa* group (*P. tomentasa, P. fargesii, P. albiprhlaca, P. lamproprhylia* and *P. elongata*) accordance to the results of the single and two-dimensional SDS-PAGE of protein in the *Paulownia* tree leaves. The result could lay a foundation for classifying the Genus *Paulownia* plants.

Key words: *Paulownia*; Leaf; Electrophoresis; Protein; Classification
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Introduction

There are many kinds of species in the Genus *Paulownia*. Chen, Westfall, Wang *et al.* have done much work about the classification of those plants in morphology and cytology since the Genus *Paulownia* was setup by Siebold and Zuccarini in 1835 (Chen 1981; Chen 1986; Darlingtong 1955; Gong 1976; Hu 1959; Gong 1994; Hu 1995; Jiang 1990; Liang 1997; Sun 1990; Liang 1997; Xiong 1992; Tong 1980; Shu 1987; Xiong 1991; Yu 1987). But there existed some disputations on the Genus classification (Chen 1986; Hu 1959; Gong 1976; *Paulownia* investigation group of Forestry Science Institution of China 1980; Xiong 1991; Sun 1990; Gong 1994; Hu 1995; Liang 1997; Xiong 1992). As the development of science and technology, some methods facilitate the illumination of the relationship among the plants in the Genus *Paulownia* (Darlingtong 1955; Liang 1997; Lin 1990; Mosseter 1992; Palmer 1987; Shu 1987; Tong 1980; Wang 1994; Yu 1987). In this paper the problem is studied with single and two-dimensional SDS-PAGE of protein in the *Paulownia* plant leaves.

Materials and methods

Materials

The fresh leaves of *P. tomentosa* (Thunb.) Steud, *P. fortunei* (Seem) Hemsl, *P. elongata* S.Y Hu f. alba Z.X.Chang et S.L.Shi, *P. lamprophylla* Z. X. Chang et S.L. Shi, *P. albiphloea* Z.H Zhu, *P. australis* Gong Tong, *P. albiphloea* var *chenggtuensis* Z.H.Zhu, *P. fargesii* Franch. and *P. elongata* S.Y.Hu collected from 5 year trees planted in 1997 in the Forestry Station of Henan Agricultural University were wrapped with gauze after being washed with tap water and distilled water immediately and put in the liquid nitrogen.

Methods

Protein samples prepared and their electrophoresis (Fan *et al.* 1997).

SDS-PAGE gel scanning

Scan with double points scanner CS-9 000 at 575 nm wavelengths.

Data analysis

After scanning the gels, the protein peaks were marked with number from 1 to 75 according to their corresponding places respectively. If there appears protein peak during scanning gels, all of them were stated with"1", otherwise with "0". For the two dimensional SDS-PAGE of proteins, those appeared protein dots stated with "1", and those disappeared dots with "0" on the given area. Data were analyzed with Simqual in the NT-SYS computer program for the similar coefficient among *Paulownia* plants. Then *Paulownia* plants were clustered and tree maps were gotten with Unweight Pair Group Method Arithmetic Clustering (UPGMAC).

Results and analysis

Single SDS-PAGE of proteins in different *Paulownia* tree leaves

Protein diversity of plant leaves of 9 Paulownia spe-

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Biography: FAN Guo-qiang (1964-), male, Professor in Institute of *Paulownia* Henan Agriculture University. Zhengzhou 450002, P. R. China.
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cies

The scanning diagram of proteins in leaves of 9 species of *Paulownia* plants indicates that their differences are very obvious. Some protein bands among the different species of *Paulownia* plant leaves, for example, 1, 4, 6, 7, 8, 14, 18, 21, 23, 25, 28, 29, 32, 35, 36, 40, 46, 53, 57, 59, 62, 65, 69, 71, 73 are the same; others are different, eg. band 74 only belonging to *P. albiphloea* var *chengtouensis*, 34 to *P. australis*, 22 to *P. fargesii*, 48 and 49 to *P. lamprophylla*, 39 and 72 to *P. albiphloea*, 55, 56 and 58 to *P. elongata*, 3, 26, 27 to *P. elongata f. alba*. *P. fortunei* and *P. tomentosa* plant leaves have not any their own special protein bands, that is to say, they may have some genetic relationship with others respectively. From the above analysis, protein diversity plant leaves of 9 *Paulownia* species may be drawn.

Similar coefficient of single SDS-PAGE of proteins in the different Paulownia plant leaves

The similar coefficient matrix of the protein SDS-PAGE of tree leaves of 9 *Paulownia* species (Table 1) shows that the protein similar coefficient in different *Paulownia* plant leaves is various (from 0.739 to 0.886).

Table 1. Similar coefficient matrix of single SDS-PAGE of proteins in tree leaves of 9 *Paulownia* species

C1	1.000								
C2	0.800	1.000							
C3	0.769	0.882	1.000						
C4	0.776	0.805	0.841	1.000					
C5	0.835	0.817	0.830	0.886	1.000				
C6	0.739	0.809	0.800	0.809	0.800	1.000			
C7	0.769	0.774	0.787	0.841	0.851	0.863	1.000		
C8	0.845	0.808	0.820	0.830	0.860	0.832	0.860	1.000	
C9	0.879	0.817	0.766	0.773	0.830	0.779	0.787	0.840	1.000

The biggest coefficient is 0.886 between *P. tomentosa* and *P. fargesii,* and the smallest one is 0.739 between *P. fortunei* and *P. lamprophylla*. The coefficient between *P. albiphloea* var *Chengtouensis* and *P. australis* is over 0.88, the one between *P. albiphloea* and *P. elongata*, *P. fortunei* and *P. elongata f. alba*, *P. fargesii* and *P. elongata* is over 0.86 respectively. This means that genetic relation between *P. tomentosa* and *P. fargesii* is the closest among the 9 species and the genetic relation between *P. fortunei* and *P. lamprophylla* is the farthest.

Cluster

The tree map (Fig. 1)of similar coefficient of single SDS-PAGE of proteins in plant leaves of 9 *Paulownia* species shows that those plants may be divided into three groups at similar coefficient 0.82: group I (*P. fortunei* and *P. elongata f. alba.*), group II (*P. albiphloea* var *Chengtouensis* and *P. australis*), group III(*P. tomentosa, P. fargesii, P. lamprophylla, P. albiphloea* and *P. elongata*). Moreover, the bigger similar coefficient (0.86) between *P. fortunei* and *P. elongata f. alba.* among plants of 9 species demonstrates that the genetic relations is close. This is because that *P. elongata f.alba.* is the crossbreed of *P. fortunei* and *P. elongata* on gene background, and that *P. fortunei* and *P. elongata* are located in the Southern area geographically. In group II, *P. australis* and *P. albiphloea* var *Chengtouensis* are distributed in Hubei and Sichuan areas, there exists resemblance between them through gene excursion. So is group III.

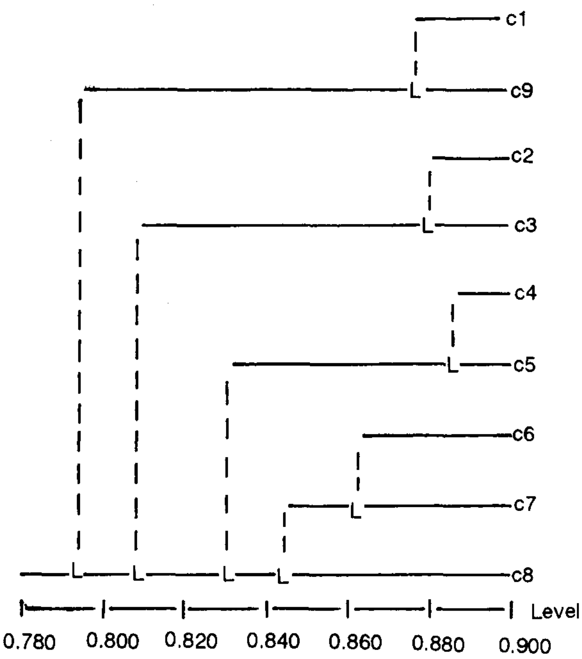

Fig. 1. Tree map of similar coefficient of single SDS-PAGE of protein in plant leaves of 9 *Paulownia* species

C1: *P. fortunei* C2: *P. albiphloea* var *Chengtouensis* C3: *P. australis* C4: *P. tomentosa* C5: *P. fargesii* C6: *P. lamprophylla* C7: *P. albiphloea* C8: *P. elongata* C9: *P. elongata f. alba.*

Two dimensional SDS-PAGE of proteins in plant leaves of 9 *Paulownia* species

Similar coefficient of two dimensional SDS-PAGE of protein in the different Paulownia plant leaves

There are many kinds of proteins or polypeptides on the two dimensional SDS-PAGE gels of tree leaves of 9 *Paulownia* species. For convenience, proteins or polypeptides with obvious changing area on the gel are focused with the method mentioned above. The similar coefficient matrix of two dimensional SDS-PAGE of proteins in plant leaves of 9 *Paulownia* "species" (Table 2) indicates that the coefficient changes from 0.809 to 0.980.

The biggest coefficient is 0.980 between *P. fortunei*

and *P. elongata f. alba.*, the smallest one is 0.809 between *P. tomentosa* and *P. fortunei*. It is clear that the genetic relation between *P.* fortunei and *P. elongata f.alba* is the closest and the one between *P. tomentosa* and *P. fortunei* is on the contrary among 9 species plants. Because the coefficients between *P. albiphloea* var *Chengtouensis* and *P. australis*, *P. lamprophylla* and *P. albiphloea*, *P. lamprophylla* and *P. fargesii* are all above 0.97, the genetic relations between them are closer.

Table 2. The coefficient matrix of two dimensional SDS-PAGE of proteins in 9 species of *Paulownia* tree leaves

C1	1.000								
C2	0.980	1.000							
C3	0.894	0.913	1.000						
C4	0.870	0.889	0.977	1.000					
C5	0.840	0.857	0.936	0.913	1.000				
C6	0.809	0.909	0.909	0.930	0.936	1.000			
C7	0.851	0.870	0.909	0.930	0.936	0.955	1.000		
C8	0.875	0.894	0.889	0.909	0.917	0.933	0.978	1.000	
C9	0.857	0.875	0.913	0.933	0.939	0.957	0.957	0.979	1.000

Cluster

On the basis of the tree map (Fig. 2) of similar coefficient of two dimensional SDS-PAGE of proteins in plant leaves of 9 *Paulownia* species, the plants may be divided into three groups at the coefficient 0.92. Group I (*P. fortunei* and *P. elongata f.alba*), group II (*P. albiphloea* var *Chengtouensis* and *P. australis*) and group III (*P. tomentosa*, *P. fargesii, P. lamprophylla, P. albiphloea* and *P. elongata*). This result is in line with one of single SDS-PAGE totally. What is more, the cluster with two dimensional SDS-PAGE of proteins in those plant leaves is more accurate than that with single SDS-PAGE, so it can more reveal their genetic relationship among those plants for the larger similar coefficient.

Discussion

Genus *Paulownia* plants were divided into *P. tomentosa, P. fortunei* and *P. taiwanensis* groups according to their flower and fruit morphology (Hu 1959). Later, 12 species (varieties) of the Genus *Paulownia* plants were classified into *P. tomentosa, P. fortunei* and *P. catalpifolia* groups by Xiong Jinqiao(1992) with systematic cluster on the basis of their 38 of morphological characteristics, eg. leaves, flowers and fruits. But some respects were not involved such as scalar characters of leaf length and width, flowering time, fruiting change with the difference of tree age, flowering year, climatic and soil condition and management level. Liang (1997b) and his collogue categorized 9 species of those plants into *P. fargesii, P. tomentosa, P. fortunei* and *P.eastern-china* groups in light of their chromosome number. This classification is more detailed submicroscopicaly, but results may be change easily with different observers because there are more subjective factors in the observing of the length and number of the chromosomes. For instance, Tong reported that the chromosome number of *P. fortunei* is 2n=18-34(n: number of chromosome) Tong (1980) and Han, but 2n=0 was reported (Shu 1987). The reason is that the chromosome is so little that it is not easily to distinguish from the other tinted granules in tablet compressing. Ma (1997) arranged 15 species of *Paulownia* plants into *P. australis, P. fortunei* and *P. tomentosa* groups based on the RFLP of their chloroplast DNA, but the blurred bands was deleted in result statistics.

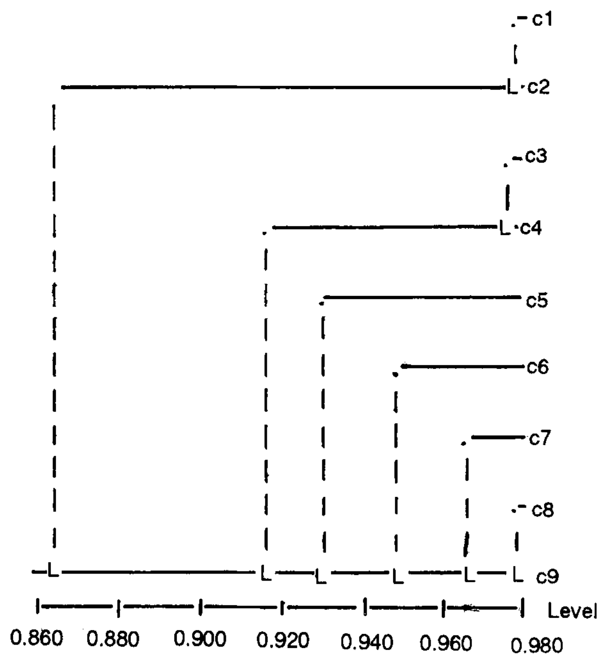

Fig. 2. Tree map of similar coefficient of two dimensional SDS-PAGE of protein in plant leaves of 9 *Paulownia* species

C1: *P. fortunei* C2: *P. albiphloea* var *Chengtouensis* C3: *P. australis* C4: *P. tomentosa* C5: *P. fargesii* C6: *P. lamprophylla* C7: *P. albiphloea* C8: *P. elongata* C9: *P. elongata f. alba.*

There exists homology and difference between the result present here and that reported by Chen and Ma (1997). *P. elongata* is a natural crossbreed of *P. fortunei* and *P. tomentosa*, and it is more related to *P. fortunei* in genetics, but its single and two-dimensional SDS-PAGE gels is close to that of *P. tomentosa*, so we put it into *P. tomentosa* group. Gong Benhai (1994) considered that *P. lamprophylla* is a crossbreed of *P. elongata* with *P. tomentosa*, which indicates that there is close genetic relation-

ship between them and provide us theoretical evidence to put *P. lamprophylla* into *P. tomentosa* group. For *P. australis* group, our result is homology with Ma's, both of us classify *P. australis* and *P. albiphloea* var *Chengtouensis* into *P. australis* group. As for *P. tomentosa* and *P. fortunei* groups, there is much coincidence between Ma Hao's result and ours (only some different ascription of species, such as, *P. lamprophylla* belonging to *P. foruntei* group in Ma Hao' paper, but to *P. tomentosa* group in ours), *P. fortunei* and *P. elongata f.albi* are classified into *P.tomentosa* group with two methods, which are consensus with Hu Xiuying and Hu Huirong (Hu 1959; Hu and Chen 1995). Because *P. lamprophylla* is confirmed to be the crossbreed of *P. tomentosa* and *P. elongata*(Hu 1959; Gong and his collogues 1994), it is more rational to put it into *P. tomentosa* group instead of *P. fortunei* group.

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